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(12) **United States Patent**  
**Miettinen-Oinonen et al.**

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(54) **CELLULASES, THE GENES ENCODING THEM AND USES THEREOF**

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(\*) Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: 08/841,636

(22) Filed: Apr. 30, 1997

(65) **Prior Publication Data**

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Related U.S. Application Data

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(60) Provisional application No. 60/005,335, filed on Oct. 17, 1995, provisional application No. 60/007,926, filed on Dec. 4, 1995, and provisional application No. 60/020,840, filed on Jun. 28, 1996.

(51) **Int. Cl.** 7 ..... C12N 9/42; C11D 3/386  
(52) **U.S. Cl.** ..... 435/204; 435/263; 510/392  
(58) **Field of Search** ..... 475/209, 263;  
510/392

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Sequence search, 1999.\*

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(57) **ABSTRACT**

Genes encoding novel cellulases, and a gene encoding a protein that facilitates the action of such novel cellulases, the novel cellulases and a protein that facilitates the action of such cellulases, and enzyme preparations containing such proteins are described. The native hosts and the culture medium of said hosts containing said novel cellulases are also disclosed. These proteins are especially useful in the textile and detergent industry and in pulp and paper industry.

16 Claims, 37 Drawing Sheets

*Claimed polypeptide  
Seq id No: 31.  
NO ODP. or DP.*

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CCGGGGACTG GCATC

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(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CCGGGGACTG CGCATC

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What is claimed is:

1. An isolated and essentially homogenous polypeptide as analyzed on SDS-PAGE having the amino acid sequence set forth in FIG. 19 (SEQ ID NO:31).  
 2. An isolated and essentially homogenous polypeptide as analyzed on SDS-PAGE having an amino acid sequence which has at least 80% identity to the amino acid sequence set forth in FIG. 19 (SEQ ID NO:31).  
 3. An enzyme preparation having cellulase activity, wherein said activity comprises the cellulase activity of a 20K polypeptide having the amino acid sequence set forth in FIG. 19 (SEQ ID NO:31), wherein said enzyme preparation is obtained by a process comprising:

- (i) culturing a host cell transformed with the nucleic acid sequence set forth in FIG. 19 (SEQ ID NO:30); and
- (ii) separating said host cell from the culture medium and obtaining the supernatant having said 20K cellulase activity;

wherein the level of 20K cellulase activity in the supernatant obtained from said transformed host cell is at least three times the level of 20K cellulase activity obtained from the supernatant of the untransformed host cell naturally expressing said cellulase when said untransformed host cell is cultivated under the same conditions.

4. An enzyme preparation having cellulase activity wherein said enzyme preparation is obtained by a process comprising:

- (i) culturing a host cell transformed with a nucleic acid sequence encoding a cellulase having at least 80% identity to the amino acid sequence of the 20K cellulase set forth in FIG. 19 (SEQ ID NO:31); and
- (ii) separating said host cell from the culture medium and obtaining the supernatant having said 20K cellulase activity or cellulase activity of a cellulase that has at least 80% identity to the sequence of said 20K cellulase set forth in FIG. 19 (SEQ ID NO:31),

wherein the level of said 20K cellulase activity or the level of said activity of said cellulase that has at least 80% identity to said sequence of said 20K cellulase set forth in FIG. 19 (SEQ ID NO:31) in the supernatant obtained from said transformed host cell is at least three times the level of said 20K cellulase activity or activity of said cellulase that has at least 80% identity to said sequence of said 20K cellulase set forth in FIG. 19 (SEQ ID NO:31), respectively, obtained from the supernatant of the strain naturally expressing said cellulase when said strain is cultivated under the same conditions.

20 5. An enzyme preparation comprising at least one cellulase from *Melanocarpus albomyces* CBS 685.95.

6. The enzyme preparation of any one of claims 3-5, which is in the form of a liquid.

7. The enzyme preparation of any one of claims 3-5, which is in the form of a dry powder.

8. A detergent composition comprising the enzyme preparation of any one of claims 3-5 in combination with a surface active agent or surfactant.

9. A method of treating cellulosic fiber containing textile material, said method comprising contacting said textile material with the detergent composition of claim 8.

10. An isolated and essentially homogenous polypeptide as analyzed on SDS-PAGE having amino acids 22-235 of the amino acid sequence set forth in FIG. 19 (SEQ ID NO:31).

35 11. An isolated and essentially homogenous polypeptide as analyzed on SDS-PAGE having an amino acid sequence which has at least 80% identity to amino acids 22-235 of the amino acid sequence set forth in FIG. 19 (SEQ ID NO:31).

12. An enzyme preparation having cellulase activity, wherein said activity comprises the cellulase activity of a polypeptide having amino acids 22-235 of the amino acid sequence set forth in FIG. 19 (SEQ ID NO:31), wherein said enzyme preparation is obtained by a process comprising:

- (i) culturing a host cell transformed with a nucleic acid sequence encoding amino acids 22-235 of the amino acid sequence set forth in FIG. 19 (SEQ ID NO:31); and

(ii) separating said host cell from the culture medium and obtaining the supernatant having said cellulase activity; wherein the level of said cellulase activity in the supernatant obtained from said transformed host cell is at least three times the level of said cellulase activity obtained from the supernatant of the strain naturally expressing said cellulase when said strain is cultivated under the same conditions.

13. An enzyme preparation having cellulase activity wherein said enzyme preparation is obtained by a process comprising:

- (i) culturing a host cell transformed with a nucleic acid sequence encoding a cellulase having at least 80% identity to amino acids 22-235 of the amino acid sequence set forth in FIG. 19 (SEQ ID NO:31); and

(ii) separating said host cell from the culture medium and obtaining the supernatant having the cellulase activity of a cellulase that has at least 80% identity to amino acids 22-235 of the amino acid sequence set forth in FIG. 19 (SEQ ID NO:31);

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wherein the level of said activity of said cellulase that has at least 80% identity to amino acids 22-235 of the amino acid sequence set forth in FIG. 19 (SEQ ID NO:31) in the supernatant obtained from said transformed host cell is at least three times the level of said activity of said cellulase that has 80% identity to amino acids 22-235 of the amino acid sequence set forth in FIG. 19 (SEQ ID NO:31) obtained from the supernatant of the strain naturally expressing said cellulase when said strain is cultivated under the same conditions.

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14. The enzyme preparation of either claim 12, or claim 13, which is in the form of a liquid.

15. The enzyme preparation of either claim 12 or claim 13, which is in the form of a dry powder.

16. A detergent composition comprising the enzyme preparation of either claim 12 or claim 13 in combination with a surface active agent or surfactant.

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